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QIAcube Automated Extraction of DNA using the QIAamp DNA Blood Mini Kit

I. Purpose

The purpose of this protocol is to provide specific guidelines for the extraction of genomic DNA from peripheral blood or Buffy Coat preparations using the QIAcube sample preparation robot.

II. Scope

All procedures are applicable to the BCGSC Library Core Group

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance team to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
QIAcube User Manual Blood and Bodily Fluid Spin Protocol V3	IM.0300 Version 3

VI. Related Documents

Document Title	Document Number
Quantifying DNA Samples using the Qubit Fluorometer QIAcube Monthly Maintenance and Cleaning	LIBPR.0030 LIBPR_Work Inst.0031

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

It is strongly recommended that any person coming in direct contact with bodily fluids, such as blood, are vaccinated against the following diseases: MMR and Hepatitis B.
Familiarize yourself with the Genome Sciences Centre Occupational Health and Safety Manual before handling any blood samples. Ensure you are familiar with the sections outlined below:

- Chapter 26 – Hazardous Waste Disposal
  - Section 26.3 – Bio-hazardous Waste
- Chapter 27 – Laboratory Ventilation and Fume Hoods
  - Section 27.6 – Biological Safety Cabinets
- Chapter 29 – Equipment Safety
  - Section 29.1 – Centrifuges
- Chapter 31 – Laboratory Bio-safety
- Appendix 3 – BCCA Blood and Body Fluid Exposure Protocol
- Appendix 4 – PHSA Blood and Body Fluid Exposure FAQ
- Appendix 13 – Hazardous Waste Disposal Echelon

Any person picking up or transporting blood from another location must possess a valid Transportation of Dangerous Goods training certificate. The certificate must be with you.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
QIAcube Robotic Workstation	Qiagen	9001292	✓	
Rotor Adapters	Qiagen	990394		✓
Filter tips	Qiagen	990352		✓
QIAcube Reagent Bottles	Qiagen	990353		✓
Safe-Lock Tubes, 2.0 mL	Eppendorf	990381		✓
2 ml screw cap sample tubes	Qiagen	990382		✓
Anhydrous Ethyl Alcohol	Commercial Alcohols	PeopleSoft ID:23878		✓
Bench Coat	Fisher	12-007-186		✓
Black Ink Marker Pen	VWR	52877-310		✓
DNA*AWAY*	MBS	7010		✓
Soft Touch Gloves	Ultident	296359683		✓
Extended cuff nitrile gloves - small	Fisher	19-149-864a		✓
Extended cuff nitrile gloves - medium	Fisher	19-149-864b		✓

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Gilson P200 Pipettor	Mandel	GF-23601		✓
Gilson P1000 Pipettor	Mandel	GF-23602		✓
Ice Bucket	Fisher	11-676-36		✓
Diamond Barrier Tips, 200 ul	Mandel	GF-F171503		✓
Diamond Barrier Tips, 1000 ul	Mandel	GF-F171703		✓
Filter Tips, 1000 ul (for QIAcube)	Qiagen	990352		✓
Filter Tips, 200 ul (for QIAcube)	Qiagen	990352		✓
Wet Ice	In House	N/A	N/A	N/A
RNase-Free DNase I Set	Sigma	D451379254		✓
QIAamp DNA Blood Mini Kit	Qiagen	51104		✓
Large Kimwipes	Fisher	06-666-117		✓
PBS	Invitrogen	10010-023		✓
T36 disinfectant	VWR	26200-152		✓
6 % bleach	Fisher	00050847		✓
Disposable transfer pipette	CW Stores	13-711-22		✓

IX. Procedure

Note this protocol is validated for the use of input materials from 200µL peripheral blood or Buffy Coat that is harvested from 2mL blood. Blood and Buffy coat should be topped up to 200µL volume with PBS if needed.

1. Introduction and Upstream Set Up

1.1. Put on a clean pair of gloves and a lab coat. Raise the sash on the QIAcube and wipe down the interior with DNA*Away* and RNaseZap. DO NOT SPRAY DIRECTLY INTO THE QIAcube in case the circuitry becomes wet. Moisten a Kimwipe with the decontaminant and wipe the surfaces. Rinse with a Kimwipe moistened with NF water. Wipe down the pipettors and bench at the work station you'll be using with DNA*Away* and RNaseZap. Lay down new bench coat and prepare an ice bucket with fresh ice.

2. Setting up the QIAcube:

2.1. Label and date five QIAcube reagent bottles (bottle and lid) with one of the following labels each:

Position	Reagent
1	–
2	Buffer AL
3	96–100% ethanol
4	Buffer AW1
5	Buffer AW2
6	Buffer AE

Figure 1 – QIAcube Reagent bottles list and loading positions

2.2. Add the required amount of absolute ethanol to AW1 and AW2 stock bottles supplied in the QIAamp DNA Blood Mini Kit. Cap the bottles and shake well to mix. Check the "added" box on the lid and date the lid. Decant from the buffer stock bottles into the matching QIAcube reagent bottles.

2.3. Load the reagent bottles into the reagents rack in the robot according to the positions shown in Figure 1. Use fresh absolute EtOH in bottle number 3 for each day. Leave the bottle lids on the bottles until you're ready to start the program.

2.4. Using Table 1 below, aliquot the appropriate volume of Proteinase K into a 1.5 mL Eppendorf tube and load into **position A** of the microcentrifuge tube slot, tucking the lid securely into the slot adjacent to the tube holder (Figure 2)

Table 1. Proteinase K volumes depending on the number of samples

	Volume of reagent required for the indicated number of samples (µl)		
Number of samples	A	B	C
2	68		
3	90		
4	111		
5	133		
6	155		
7	176		
8	198		
9	219		
10	241		
12	284		

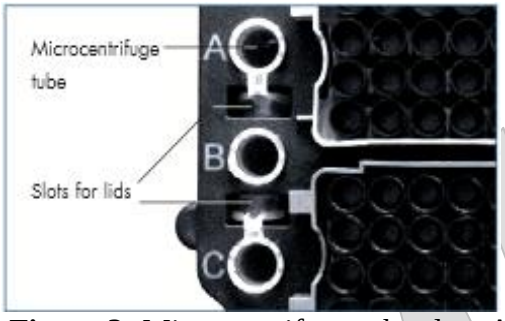


Figure 2- Microcentrifuge tube slots A, B, C

2.5. Fill one tip rack with QIAcube 1000 µl tips and the other with 200 µl tips. The robot can detect the size of tips so either position is acceptable.

Note: Each tip rack has 2 notches on each side that the optical sensor uses to identify the tip rack during the load check. When filling tip racks makes sure the 2 notches are not broken, otherwise the run will fail.

2.6. Use a small autoclave bag as a liner for the waste container. **The tips from this protocol must go into the bio-hazard waste** and making a liner makes the clean-up easier and safer.

2.7. Change the shaker adapter to the one designed for screw-cap tubes (labelled "S2"): Remove the blue plastic tube rack by gently pulling straight up. Using the hex wrench provided with the QIAcube, unscrew the two retaining screws and remove the shaker adapter. Replace it with the "S2" adapter and tighten the retaining screws. Slip the blue plastic tube rack back into position, over the adapter.

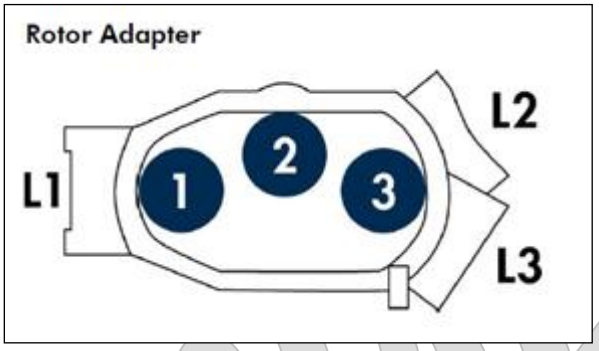
3. Centrifuge Adapters Set up:

The 1.5mL microcentrifuge tubes and columns that are loaded into the holders in the adapters must be from the Qiagen kit. The QIAcube will indicate an error and will not run if any tube or column is in the wrong position or is of the wrong type.

3.1. All 1.5mL microcentrifuge collection tubes must be labelled with the library name or number, stating that it's DNA from blood or Buffy Coat and the date. In addition, label the side of the disposable adapter with the same ID and date.

3.2. Place the 1.5mL collection tube in **position 3** of the adapter according to Figure 3 below. The lid of the DNA collection tube must be tucked down into the plastic clip L3

on the outer edge of the adapter. The collection tube must sit with the rim flush against the rim of the holder. **If it is not pushed all the way down into the holder, it could break off during centrifugation and cause the run to crash.**



Position	Labware	Lid position
1	QIAamp spin column	L1
2	–	–
3	1.5 ml collection tube*	L3

Figure 3: DNA extraction Rotor Adapter Set Up

3.3. The tabs must be cut from the lids of the spin columns so that the gripper in the robot can grab the spin columns and move them when necessary. Using a razor, cut the connector tab off as close to the rim as possible. Any piece of plastic sticking out could cause the gripper to fail when it tries to transfer the column to elution tube; that will cause the run to fail. Discard the cut lid and the collection tube provided with the column.

4. Setting up Work Space in the Biological Safety Cabinet and Making Aliquots for Extraction

4.1. Wearing gloves and a disposable lab gown, turn on the BSC and spray the surface of the BSC with T36 disinfectant and wipe down with a large Kimwipe. Ensure the blower of the BSC is running for at least 5 minutes before starting to work in the hood.

4.2. Set up a biohazard bag in the hood to be used as a solid waste container during the blood processing.

4.3. Take out the appropriate number of empty 2mL screw cap tubes to be used for the blood or Buffy Coat aliquots. Label the tubes with the appropriate pla numbers.

4.4. All blood work must be performed in the BSC. Wear extended cuff, heavy duty nitrile gloves or wear a second set of gloves over the first.

4.5. The blood aliquots should be loaded last onto the QIAcube to minimize the risk of contamination.

4.6. Confirm that the ID (Original Source name) on the label of the Vacutainer/tubes matches the original source name on the empty 2mL screw cap tubes designated for the blood aliquots.

4.7. Blood is usually provided in Vacutainer vials that have a removable and pierceable lid. Remove the Vacutainer lid by gently twisting and pulling at the same time. Thoroughly mix the blood by pipetting up and down several times using a sterile disposable transfer pipette. Discard the pipette in the bio-hazardous waste bag.

4.8. Confirm with your supervisor the volume of blood or Buffy coat needed for the extraction. Aliquot the blood or Buffy coat into each of the labelled 2mL screw cap tubes (from Step 4.3). Top up the aliquots to 200µL PBS if needed. Re-cap the blood Vacutainer. Any leftover blood should be stored in the -80C freezer.

4.9. Cap the 2 mL tubes and quick spin in the microfuge. Put all of the blood or Buffy Coat aliquots into a fresh bio-hazard bag to transport them to the QIAcube.

5. Loading the Adapters onto the Centrifuge and Loading the Samples into the Shaker Rack

It is crucial to load the centrifuge adapters carefully and in the same orientation as the protocol states (see Appendix 2).

5.1. The centrifuge rotor is labeled with positions 1 to 12. The shaker rack is also labeled with positions 1 to 12. When you are loading the centrifuge with adapters and the shaker rack with your samples, **keep the position of the source tube in the shaker matching the position of the destination adapter** (eg. source sample in position # 1 in the shaker rack has to match with the collection tube in swinging bucket # 1 in the centrifuge). In addition, the adapters must be in balance with each other within the centrifuge. Refer to the loading guide in Appendix 2 for visual instruction of how to load multiple numbers of samples and adapters.

5.2. When you are certain you are ready to start the QIAcube protocol, remove the lids from the reagent bottles. Next insert the silicon plugs into the slots next to where the blood aliquots will be placed. The program will not run unless these plugs are in place beside each sample. Finally, when you have double-checked that the robot is ready and loaded, remove and discard the lids from the blood tubes into the bio-hazard bag and place the tubes into the shaker rack next to the silicon plugs, in the order that matches the order of the adapters in the centrifuge. Lower the sash of the QIAcube.

6. QIAcube Program Setup:

6.1. Turn on the QIAcube. From the main menu, choose the "DNA" box.

6.2. On the next screen choose "QIAamp DNA Blood Mini". Hit the select button. Select "Blood or body fluid".

6.3. From the next menu, choose "Elution volume 100 µL" and hit the select button.

6.4. On the next screen choose Blood and body fluid spin protocol V3 and select start.

6.5. The next screen will show QIAamp DNA Blood Mini, Starting Material: 200 µL blood or body fluid, Elution volume 100 µL. Select the "Next" button.

6.6. Follow the prompts for preparing the robot, each time choosing "Next" after you are asked if you've completed each task eg. "empty waste drawer", "fill both tip racks". Hit start and the program will begin DNA extraction.

6.7. When the DNA extraction part of the program has completed an alarm will sound. Remove the adapters from the centrifuge and check the collection tubes visually to see that the elution has been successful. Eyeball the volume to see that the appropriate DNA volume is eluted in the collection tube (ie. 100 µL) and discard the DNA spin columns. Close the collection tubes caps and place the tubes on ice until needed for QC steps and pooling if necessary. If the elution was not successful, do not discard the filters; consult with your supervisor.

6.8. Wearing a double set of fresh gloves, discard the screw cap tubes into the bio-hazard bag. Empty the waste container (with the liner) into the bio-hazard bag. Dispose of the outer set of gloves into the bag. Seal the top and transport the bag back to the tissue culture room for disposal in the bio-hazard bin. Discard the inner set of gloves in the tissue culture room.

6.9. Put on a new pair of heavy duty gloves. Remove the blue plastic tube holder from the S2 adapter and place it in a fresh bio-hazard bag. Transport it to the Biological Safety Cabinet and remove it from the bag. Spray it thoroughly with T36 spray. Leave it for 5 minutes, then wipe dry. Rinse it with 70 % EtOH and wipe dry. Return it to the QIAcube. If necessary, switch the S2 shaker adapter back out of the QIAcube and replace it with the adapter for the 2 mL safe-lock tubes. Slip the blue tube holder back onto the adapter.

6.10. Remove all reagent bottles from the QIAcube and cap tightly.

6.11. Discard the tube that contained the Proteinase K.

6.12. Wipe down the interior of the QIAcube with DNA*Away* and RNaseZap. DO NOT SPRAY DIRECTLY INTO THE QIAcube to avoid wetting the circuitry. Spray the decontaminant onto a Kimwipe and wipe the surfaces with it. Rinse with a clean Kimwipe that's been moistened with NF water.

7. Pooling and QC of the gDNA

7.1. If there were multiple tubes of the same sample extracted, pool the matching gDNA samples into one tube. Measure the volume and record it on the side of the tube. If the volume is out by more than 10 %, consult with your APC.

7.2. To assay the yield of gDNA, make a 1:2 dilution of the gDNA and run on the Qubit Fluorometer following LIBPR.0030. Save the excel file R:\Library Core\QC\Qubit. After saving the raw data, create another column in the file, entitled "Total Yield (ng)". Using the volume of the DNA and the value of DNA in nanograms per µL, calculate the total number of nanograms and list it in the new column.

7.3. Forward the gDNA QCs and the final volumes to your supervisor for evaluation. If the quantity pass the acceptance criteria, the samples can proceed to library construction. Store the gDNA samples at 4^{o}C.

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Appendix 1. LIMS SOP:

1. QIAcube Blood Extraction

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Appendix 2: Loading Guide